CLAIMS

We Claim:

1. An enzyme-linked probe suitable for use in an *in-situ* hybridization assay and further characterized in that it comprises a probing nucleobase sequence directed to a yeast specific target sequence.
2. The probe of claim 1, wherein the target sequence is ribosomal RNA.
3. The probe of claim 1, wherein the probe is a nucleic acid.
4. The probe of claim 1, wherein the probe is a peptide nucleic acid.
5. The probe of claim 1, wherein the probing nucleobase sequence is designed to detect, identify or enumerate organisms of one or more species of yeast.
6. The probe of claim 1, wherein the probing nucleobase sequence is designed to detect, identify or enumerate organisms of one or more genus of a yeast.
7. The probe of claim 1, wherein the probing nucleobase sequence is designed to detect, identify or enumerate all yeast in a sample.
8. The probe of claim 1, wherein the enzyme is selected from the group consisting of: a polymerase, alkaline phosphatase, horseradish peroxidase and soy bean peroxidase.
9. A probe suitable for detecting, identifying or quantitating the presence of *Dekkera/Brettanomyces* yeast and particularly *Dekkera bruxellensis (Brettanomyces)* in a sample of interest.
10. The probe of claim 9, wherein the probe comprises a probing nucleobase sequence wherein at least a portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.
11. The probe of claim 10, wherein the probing nucleobase sequence is exactly as it appears in the claim.
12. The probe of claim 9, wherein the probe is a peptide nucleic acid.
13. The probe of claim 9, wherein the probe is unlabeled.
14. The probe of claim 9, wherein the probe is labeled with a detectable moiety.

15. The probe of claim 14, wherein the detectable moiety is selected from the group consisting of: chromophores, fluorophores, spin labels, radioisotopes, enzymes, haptens and chemiluminescent compounds.

16. The probe of claim 15, wherein the probe is labeled with soy-bean peroxidase.

17. The probe of claim 15, wherein the probe is a probe labeled with one or more fluorophores.

18. The probe of claim 9, wherein the probe is support bound.

19. The probe of claim 18, wherein the probe exists attached to an array of probes.

20. A probe set suitable for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in a sample of interest.

21. The probe set of claim 20, wherein one or more of the probes specific for *Dekkera/Brettanomyces* yeast comprise a probing nucleobase sequence wherein at least portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

22. The probe set of claim 21, wherein the probing nucleobase sequences are exactly as represented in the claim.

23. The probe set of claim 20, wherein the probe set is specific for both the detection of *Dekkera/Brettanomyces* yeast as well as other organisms of interest in the same sample and in the same assay.

24. The probe set of claim 23, wherein the different probes of the set are independently detectable.

25. The probe set of claim 20, wherein some of the probes of the set are blocking probes.

26. The probe set of claim 20, wherein all probes of the set are peptide nucleic acids.

27. The probe set of claim 20, wherein probes of the set are labeled with a detectable moiety.

28. The probe set of claim 27, wherein the detectable moiety is selected from the group consisting of: chromophores, fluorophores, spin labels, radioisotopes, enzymes, haptens and chemiluminescent compounds.

29. The probe set of claim 28, wherein the probes are labeled with the enzyme soy-bean peroxidase.

30. The probe set of claim 28, wherein the probes are labeled with one or more fluorophores.

31. The probe set of claim 26, wherein all probes of the set are unlabeled.

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32. The probe set of claim 20, wherein the probes are support bound.

33. A probe set suitable for detecting, identifying or quantitating *Dekkera bruxellensis* yeast in a sample of interest.

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34. The probe set of claim 33, wherein the two or more probes specific for *Dekkera bruxellensis* yeast comprise a probing nucleobase sequence wherein at least portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5) and CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6) and sequences fully complementary thereto and of the same length.

35. The probe set of claim 34, wherein the probing nucleobase sequences are exactly as represented in the claim.

36. The probe set of claim 34, wherein the probe set is specific for both the detection of *Dekkera bruxellensis* yeast as well as other organisms of interest in the same sample and in the same assay.

37. The probe set of claim 36, wherein the different probes of the set are independently detectable.

38. The probe set of claim 33, wherein some of the probes of the set are blocking probes.

39. The probe set of claim 33, wherein all probes of the set are peptide nucleic acids.

40. The probe set of claim 33, wherein probes of the set are labeled with a detectable moiety.

41. The probe set of claim 40, wherein the detectable moiety is selected from the group consisting of: chromophores, fluorophores, spin labels, radioisotopes, enzymes, haptens and chemiluminescent compounds.

42. The probe set of claim 41, wherein the probes are labeled with the enzyme soy-bean peroxidase.

43. The probe set of ~~claim~~ 41, wherein the probes are labeled with one or more fluorophores.

44. The probe set of claim 39, wherein all probes of the set are unlabeled.

45. The probe set of claim 33, wherein the probes are support bound.

46. A method for detecting, identifying or enumerating yeast in a sample of interest, said method comprising:

a) contacting one or more species of yeast in the sample with one or more yeast specific enzyme-linked probes, under suitable *in-situ* hybridization conditions, to thereby form one or more probe/target sequence hybrids within the yeast; and

b) detecting enzyme activity within the yeast to thereby determine the presence, absence or number of yeast sought to be detected in the sample.

47. The method of claim 46 further comprising the step of:

c) isolating the yeast using a filter as an isolation medium.

48. The method of claim 47, further comprising the step of:

d) growing the isolated yeast by culture in a suitable media.

49. The method of claim 48, wherein the culture is grown directly on the filter, under suitable culture conditions, by placing the filter in contact with suitable media.

50. The method of claim 49, wherein colonies grown on the filter represent the number of colony forming units (CFU) present in the sample.

51. The method of claim 48, wherein the yeast are slow growing yeasts.

52. The method of claim 51, wherein the identity and number of slow growing yeasts in the culture is determined within 48 hours or less.

53. The method of claim 46, wherein the target sequence is ribosomal RNA.

54. The method of claim 53, wherein the ribosomal RNA target sequence is specific for detecting *Dekkera/Brettanomyces* yeast and particularly *Dekkera bruxellensis* (*Brettanomyces*) in a sample.

55. The method of claim 46, wherein the one or more yeast specific enzyme-linked probes are designed to detect a particular species of yeast.

56. The method of claim 46, wherein the one or more yeast specific enzyme-linked probes are designed to detect members of a genus of a yeast.

57. The method of claim 46, wherein the one or more yeast specific enzyme-linked probes are designed to detect all yeast present in the sample.

58. The method of claim 46, wherein the enzyme-linked probe is an enzyme-linked peptide nucleic acid probe.

59. The method of claim 58, wherein the probe is a soy bean peroxidase labeled peptide nucleic acid probe.

60. A method for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in a sample; said method comprising:

a) contacting one or more species of yeast in the sample with one or more *Dekkera/Brettanomyces* yeast specific probes, under suitable hybridization conditions, to thereby form a probe/target sequence hybrid; and

b) detecting the presence, absence or amount of probe/target sequence hybrid and correlating the result with the presence, absence or number of *Dekkera/Brettanomyces* yeast in the sample.

61. The method of claim 60, wherein one or more of the *Dekkera/Brettanomyces* yeast specific probes comprises a probing nucleobase sequence wherein at least portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-

GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

62. The method of claim 61, wherein the probing nucleobase sequences are exactly as represented in the claim.

63. The method of claim 60, wherein the assay is an *in-situ* hybridization assay.

64. The method of claim 60, wherein the one or more *Dekkera/Brettanomyces* yeast specific probes are labeled with a detectable moiety.

65. The method of claim 64, wherein the detectable moiety is selected from the group consisting of: chromophores, fluorochromes, spin labels, radioisotopes, enzymes, haptens and chemiluminescent compounds.

66. The method of claim 65, wherein the probes are labeled with the enzyme soy-bean peroxidase.

67. The method of claim 60, wherein the probes are labeled with one or more fluorophores.

68. The method of claim 60, wherein the method specifically detects the presence, absence or number of *Dekkera bruxellensis* yeast in the sample.

69. The method of claim 60, wherein the method detects *Dekkera/Brettanomyces* yeast as well as other organisms of interest that may be present in the sample.

70. The method of claim 69, wherein the probes suitable for detecting the different organisms in the sample are each labeled with independently detectable moieties.

71. The method of claim 70, wherein the independently detectable moieties are independently detectable fluorophores.

72. A kit suitable for performing an assay that detects, identifies or enumerates *Dekkera/Brettanomyces* yeast in a sample, wherein said kit comprises:

a) one or more *Dekkera/Brettanomyces* specific probes; and

b) other reagents or compositions necessary to perform the assay.

73. The kit of claim 72, wherein the *Dekkera/Brettanomyces* specific probe is labeled with a detectable moiety.

74. The kit of claim 72, wherein two or more probes of the kit are labeled with independently detectable moieties.

75. The kit of claim 74, wherein the independently detectable moieties are used to distinguish between the different organisms sought to be detected in the same sample and in the same assay.

76. The kit of claim 72, wherein the two or more independently detectable moieties are each independently selected from the group consisting of: chromophores, fluorochromes, spin labels, radioisotopes, enzymes, haptens and chemiluminescent compounds.

77. The kit of claim 72, wherein hybridization of the probing nucleobase sequence of the probe to the target sequence is detected using an antibody or antibody fragment, wherein the antibody or antibody fragment specifically binds to the peptide nucleic acid/nucleic acid complex formed under antibody binding conditions.

78. The kit of claim 77, comprising an antibody labeled with a detectable moiety.

79. The kit of claim 72, wherein the assay is an *in-situ* hybridization assay.

80. The kit of claim 72, comprising:

a) a filter for isolating yeast from a sample of interest;

b) culture media for growing the isolated yeast;

c) fixation solution for fixing grown yeast;

d) a hybridization solution suitable for imposing suitable hybridization conditions;

e) a soy bean peroxidase labeled probe specific for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in the sample;

f) one or more wash solutions for removing undesirable components after performing one or more steps of the assay; and optionally

g) an enzyme substrate suitable for generating detectable signal from the enzyme activity of the soy bean peroxidase linked to the peptide nucleic acid probe; or

h) a film for detecting signal generated from the enzyme activity.

81. The kit of claim 80, wherein the fixation solution and the hybridization solution are the same solution.

82 The kit of claim 80, wherein the soy bean peroxidase labeled probe is a peptide nucleic acid.

83. A method for quantitating slow growing yeast in a liquid sample in less than 48 hours; said method comprising:

a) filtering a fixed volume of liquid using a filter having a pore size that does not allow the yeast to pass;

b) incubating the filter containing the yeast, in a suitable culture media and under suitable culture conditions, for 45 or fewer hours;

c) fixing the microcolonies of yeast to the filter;

d) contacting the microcolonies of yeast with a yeast specific enzyme-linked probe, under suitable *in-situ* hybridization conditions to thereby form one or more probe/target sequence hybrids within the yeast;

e) detecting enzyme activity within the yeast to thereby determine the presence, absence or number of yeast sought to be detected in the sample; and

f) determining the quantity of yeast in the sample.

84. The kit of claim 83, wherein fixing the microcolonies of yeast to the filter and contacting the microcolonies of yeast with a yeast specific enzyme-linked probe are performed simultaneously using a single solution.

85. The kit of claim 83, wherein the number of CFU in the sample is determined.